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Abstract. In this paper, we improve and extend the approach of Wang and Xia for stability analysis of biological systems by making use of Gröbner bases, (CAD-based) quantifier elimination, and discriminant varieties, as well as the stability criterion of Liénard and Chipart, and showing how to analyze the stability of Hopf bifurcation points. The stability and bifurcations for a class of self-assembling micelle systems with chemical sinks are analyzed in detail. We provide experimental results with comparisons for 15 biological models taken from the literature.

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1. Introduction

Many biological networks can be modeled by dynamical systems. Only few nonlinear dynamical systems have exact analytic solutions, so qualitative analysis of stability, bifurcations, and chaos becomes a primary means for us to study their behaviors. The analysis is highly nontrivial and for it a whole body of theory and sophisticated methods has been developed in mathematics. Such methods are associated with the names of many great mathematicians including A. M. Liapunov, H. Hopf, J. H. Poincaré, and V. I. Arnold. The methods have been widely used both for theoretical studies and in experimental investigations in many areas where dynamical systems are involved.

The qualitative behaviors of dynamical systems may be observed experimentally by means of numerical simulation and visualization, or studied rigorously by

means of symbolic computation and formal reasoning. The numerical approach has been used extensively in the literature of experimental biology [2, 5, 10, 31, 33], control theory, and other areas of engineering, while the symbolic approach involves complicated algebraic calculations and derivations and its application is still at the beginning of research (see, e.g., [1, 13, 17, 46]).

It is an important issue to detect the equilibria of a biological system and to analyze the stability of each equilibrium, in order to see whether the state of the system will move away from or return to one of the stable equilibria (or limit cycles, or attractors) in response to a perturbation. In [46], Wang and Xia proposed a general approach for the detection and stability analysis of real equilibria for a class of biological systems by means of symbolic and algebraic computation. They have successfully analyzed the stability of several biological systems using their approach and a software program of real solution classification [46, 47].

The main algebraic tools used in the approach described in [46] are the methods of triangular decomposition [43, 49] and real solution classification [52]. It turns out that other methods based on Gröbner bases [8], resultants [14, 43], cylindrical algebraic decomposition (CAD) [11, 12], quadratic quantifier elimination [48], and discriminant varieties [25] for variable elimination and real solving may also be applied to the same problem of stability analysis. Moreover, the approach may be generalized to analyze the bifurcation of limit cycles, hysteresis, oscillation, and other phenomena of biological systems. Such algebraic analysis allows one to track and understand how the equilibrium solutions of a biological system change as one or more parameters vary. Relevant work in this direction has been done, e.g., by Hong, El Kahoui, Anai, and others in [17, 13, 1] where quantifier elimination is applied to stability tests, Hopf bifurcation analyses, and other problems in biology and by Chen in [9] where the approach of Wang and Xia is investigated and improved.

In this paper, we further improve and extend the approach of Wang and Xia by making use of Gröbner bases [8, 15], (CAD-based) quantifier elimination [11, 12], and discriminant varieties [25], as well as the stability criterion of Liénard and Chipart [26], and showing how to analyze the stability of Hopf bifurcation points of two-dimensional systems. The paper is structured as follows. In the following section, we explain how to reduce the problem of stability analysis for a large class of biological systems to purely algebraic problems. The stability criteria of Routh–Hurwitz and Liénard–Chipart are provided in Section 3. Several well-known algebraic methods are reviewed briefly in Section 4 and then used in Section 5 to deal with the algebraic problems formulated from stability analysis. In Section 6, we show how bifurcation analysis may also be carried out by using algebraic methods. Section 7 presents the application of the methods of CAD and discriminant varieties to stability analysis. In Section 8, the stability and bifurcations for a class of self-assembling micelle systems with chemical sinks are analyzed in detail. Section 9 contains experimental results with timing statistics and comparisons on stability analysis for 15 biological models taken from the literature (and listed in the appendix). The paper is concluded with a few remarks.

2. Reduction of stability analysis to algebraic problems

We consider biological networks that may be modeled by autonomous systems of ordinary differential equations of the form

$$\begin{cases} \dfrac{\mathrm{d}x_1}{\mathrm{d}t} = \dfrac{P_1(\lambda_1,\ldots,\lambda_m,x_1,\ldots,x_n)}{Q_1(\lambda_1,\ldots,\lambda_m,x_1,\ldots,x_n)}, \\ \cdots\cdots \\ \dfrac{\mathrm{d}x_n}{\mathrm{d}t} = \dfrac{P_n(\lambda_1,\ldots,\lambda_m,x_1,\ldots,x_n)}{Q_n(\lambda_1,\ldots,\lambda_m,x_1,\ldots,x_n)}, \end{cases} \tag{2.1}$$

where $P_1,\ldots,P_n,Q_1\neq 0,\ldots,Q_n\neq 0$ are polynomials in $\lambda_1,\ldots,\lambda_m,x_1,\ldots,x_n$ with integer coefficients and $\lambda_1,\ldots,\lambda_m$ are real parameters independent of the derivation variable t. As usual, each x_i is a function of t, and sometimes we write $\dot{x}_i$ instead of $\mathrm{d}x_i/\mathrm{d}t$. Let $\boldsymbol{\lambda}=(\lambda_1,\ldots,\lambda_m)$, $\boldsymbol{x}=(x_1,\ldots,x_n)$ and denote by $\mathbf{R}$ the field of real numbers. For any given real values $\bar{\boldsymbol{\lambda}}$ of the parameters $\boldsymbol{\lambda}$, a point $\bar{\boldsymbol{x}}$ in the n-dimensional real space $\mathbf{R}^n$ is called a *steady state* (or an *equilibrium*, or a *singular point*, or a *critical point*) of (2.1) if $\boldsymbol{x}=\bar{\boldsymbol{x}}$ is a common real solution of $P_1=\cdots=P_n=0$, $Q_1\cdots Q_n\neq 0$, i.e., $\bar{\boldsymbol{x}}\in\mathbf{R}^n$ such that

$$P_1(\bar{\boldsymbol{\lambda}},\bar{\boldsymbol{x}})=\cdots=P_n(\bar{\boldsymbol{\lambda}},\bar{\boldsymbol{x}})=0,\quad Q_1(\bar{\boldsymbol{\lambda}},\bar{\boldsymbol{x}})\cdots Q_n(\bar{\boldsymbol{\lambda}},\bar{\boldsymbol{x}})\neq 0.$$

Form the following system of polynomial equations and inequations

$$\begin{aligned} &P_1(\boldsymbol{\lambda},\boldsymbol{x})=0,\ldots,P_n(\boldsymbol{\lambda},\boldsymbol{x})=0,\\ &Q_1(\boldsymbol{\lambda},\boldsymbol{x})\neq 0,\ldots,Q_n(\boldsymbol{\lambda},\boldsymbol{x})\neq 0. \end{aligned} \tag{2.2}$$

Then the general problem of determining the (number of) steady states of (2.1) is reduced to the following two algebraic problems (as stated in [46]).

Problem 1. Assume that the parameters $\boldsymbol{\lambda}$ are not present. Determine the number of real solutions of (2.2) for $\boldsymbol{x}$ and isolate all the isolated real solutions of (2.2) by rational intervals.

Problem 2. For any integer $k\geq 0$, determine the conditions on $\boldsymbol{\lambda}$ for system (2.2) to have exactly k distinct real solutions for $\boldsymbol{x}$.

An isolated steady state $\bar{\boldsymbol{x}}$ of (2.1) (for fixed real parametric values $\bar{\boldsymbol{\lambda}}$) is said to be *stable* if for every $\epsilon>0$ and any $t_0>0$ there exists a $\delta(\epsilon)>0$ such that $\|\boldsymbol{x}(t)-\bar{\boldsymbol{x}}\|<\epsilon$ for all $t\geq t_0$, whenever $\|\boldsymbol{x}(t_0)-\bar{\boldsymbol{x}}\|<\delta(\epsilon)$. In other words, $\bar{\boldsymbol{x}}$ is stable if all the solutions of (2.1) that start "sufficiently close" to $\bar{\boldsymbol{x}}$ stay "close" to $\bar{\boldsymbol{x}}$. We want to analyze the stability of each isolated steady state of (2.1) and to determine conditions on the parameters for isolated steady states to be stable. For this purpose, we use the first method of Lyapunov with the technique

of linearization. Consider the $n \times n$ Jacobian matrix

$$\boldsymbol{J}(\boldsymbol{\lambda}, \boldsymbol{x}) = \begin{bmatrix} \frac{\partial \frac{P_1}{Q_1}}{\partial x_1} & \cdots & \frac{\partial \frac{P_1}{Q_1}}{\partial x_n} \\ \vdots & & \vdots \\ \frac{\partial \frac{P_n}{Q_n}}{\partial x_1} & \cdots & \frac{\partial \frac{P_n}{Q_n}}{\partial x_n} \end{bmatrix}.$$

For each isolated steady state $\bar{\boldsymbol{x}}$ with real parametric values $\bar{\boldsymbol{\lambda}}$, system (2.1) may be written in the following matrix form:

$$\dot{\boldsymbol{x}}^{\mathrm{T}} = \boldsymbol{J}(\bar{\boldsymbol{\lambda}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}} + \boldsymbol{G},$$

where the superscript T denotes matrix transpose and

$$\boldsymbol{G} = \left[\frac{P_1(\bar{\boldsymbol{\lambda}}, \boldsymbol{x})}{Q_1(\bar{\boldsymbol{\lambda}}, \boldsymbol{x})}, \ldots, \frac{P_n(\bar{\boldsymbol{\lambda}}, \boldsymbol{x})}{Q_n(\bar{\boldsymbol{\lambda}}, \boldsymbol{x})}\right]^{\mathrm{T}} - \boldsymbol{J}(\bar{\boldsymbol{\lambda}}, \bar{\boldsymbol{x}})(\boldsymbol{x} - \bar{\boldsymbol{x}})^{\mathrm{T}}$$

is $o(|\boldsymbol{x} - \bar{\boldsymbol{x}}|)$ as $\boldsymbol{x} \to \bar{\boldsymbol{x}}$. The following theorem serves to determine the stability of the steady state $\bar{\boldsymbol{x}}$.

Theorem 1 ([28, Theorem 5.5]). (*a*) If all the eigenvalues of the matrix $\boldsymbol{J}(\bar{\boldsymbol{\lambda}}, \bar{\boldsymbol{x}})$ have negative real parts, then $\bar{\boldsymbol{x}}$ is asymptotically stable.

(*b*) If the matrix $\boldsymbol{J}(\bar{\boldsymbol{\lambda}}, \bar{\boldsymbol{x}})$ has at least one eigenvalue with positive real part, then $\bar{\boldsymbol{x}}$ is unstable.

A univariate polynomial A with real coefficients is said to be *stable* if the real parts of all the roots of A are negative. In particular, let

$$A = a_0\lambda^n + a_1\lambda^{n-1} + \cdots + a_n \tag{2.3}$$

be the characteristic polynomial of $\bar{\boldsymbol{J}} = \boldsymbol{J}(\bar{\boldsymbol{\lambda}}, \bar{\boldsymbol{x}})$. The eigenvalues of $\bar{\boldsymbol{J}}$ are simply the roots of the polynomial A for λ, so if A is stable, then $\bar{\boldsymbol{x}}$ is stable. If some of the eigenvalues have zero real parts, but none of them has a positive real part, then the analysis of stability of $\bar{\boldsymbol{x}}$ becomes more difficult. In this case, if every eigenvalue with zero real part corresponds to a simple zero of A, then $\bar{\boldsymbol{x}}$ is stable; otherwise, it may be unstable (see [28]). When $\bar{\boldsymbol{J}}$ has a zero eigenvalue, the determinant of $\bar{\boldsymbol{J}}$ is 0 (i.e., $\bar{\boldsymbol{J}}$ is singular) and thus 0 is a zero of A. This case is known as of *fold bifurcation* [21]. The case in which $\bar{\boldsymbol{J}}$ has a pair of purely imaginary eigenvalues is known as of *Hopf bifurcation*. In both cases, $\bar{\boldsymbol{x}}$ is called a *bifurcation point*. In Section 6, we will explain how to treat the difficult case of Hopf bifurcation for $n = 2$.

To analyze the stability of $\bar{\boldsymbol{x}}$ according to Theorem 1 (a), we need to determine whether all the eigenvalues of $\bar{\boldsymbol{J}}$ have negative real parts. This can be done by using the stability criteria of Routh–Hurwitz [22, pp. 478–482] and Liénard–Chipart [26] described in the following section. These criteria reduce the problem of determining the negative signs of the real parts of the eigenvalues of $\bar{\boldsymbol{J}}$ to the

problem of determining the signs of certain coefficients a_i of A and the signs of certain determinants Δ_j of matrices with a_i or 0 as entries.

Now let $H_1, \ldots, H_r$ be polynomials in $\boldsymbol{\lambda}$ and $\boldsymbol{x}$ with rational coefficients. In practice, H_i may take some of the a_i and Δ_j mentioned above. Then the problem of stability analysis according to Theorem 1 (a) is reduced to that of determining the signs of H_i at the steady states for given parametric values and to establish the conditions on the parameters $\boldsymbol{\lambda}$ for H_i to be 0, positive, or negative at the prescribed number of steady states. Note that in general the real value of a steady state $\bar{\boldsymbol{x}}$ cannot be exactly computed, so one cannot simply evaluate the values of H_i at $\boldsymbol{x} = \bar{\boldsymbol{x}}$. Therefore, we arrive at the following algebraic problems for the stability analysis of (2.1), which were formulated first in [46].

Problem 3. Assume that the parameters $\boldsymbol{\lambda}$ are not present. Determine the signs of $H_1, \ldots, H_r$ at each isolated real solution of (2.2).

Problem 4. Determine the conditions on $\boldsymbol{\lambda}$ for $H_1, \ldots, H_r$ to be 0, positive, or negative at the (prescribed number of) isolated real solutions of (2.2).

3. Routh–Hurwitz and Liénard–Chipart criteria

Let A be a real polynomial in λ as in (2.3) and assume that $a_0 > 0$ (if $a_0 < 0$, then A may be scaled by -1, which does not change the zeros of A).

Define the $n \times n$ matrix

$$H = \begin{bmatrix} a_1 & a_3 & a_5 & \cdots & a_{2n-1} \\ a_0 & a_2 & a_4 & \cdots & a_{2n-2} \\ 0 & a_1 & a_3 & \cdots & a_{2n-3} \\ 0 & a_0 & a_2 & \cdots & a_{2n-4} \\ 0 & 0 & a_1 & \cdots & a_{2n-5} \\ \vdots & \vdots & \vdots & & \vdots \end{bmatrix}, \tag{3.1}$$

where $a_i = 0$ for $i > n$. H is called the *Hurwitz matrix* associated with A. Let $\Delta_1, \Delta_2, \ldots, \Delta_n$ be the leading principal minors of H, known as the *Hurwitz determinants* of A.

Theorem 2 (Routh–Hurwitz Criterion). The polynomial A is stable if and only if

$$\Delta_1 > 0,\ \Delta_2 > 0, \ldots, \Delta_n > 0. \tag{3.2}$$

This stability criterion, due to Hurwitz [18] and Routh [35], is well known as Routh–Hurwitz's stability criterion [22, 28]. It has been widely used for stability analysis.

Expanding Δ_n along the last column, one can easily see that $\Delta_n = a_n \Delta_{n-1}$. It follows that the condition $\Delta_{n-1} > 0$, $\Delta_n > 0$ is equivalent to $\Delta_{n-1} > 0$, $a_n > 0$. Therefore, condition (3.2) is equivalent to

$$\Delta_1 > 0,\ \Delta_2 > 0, \ldots, \Delta_{n-1} > 0,\ a_n > 0. \tag{3.3}$$

In other words, Δ_n in (3.2) may be replaced by a_n.

Liénard and Chipart [26] streamlined the stability criterion of Routh–Hurwitz, showing that only about half of the Hurwitz determinants are indeed needed and the remaining Hurwitz determinants may be replaced by certain coefficients a_i of A.

Theorem 3 (Liénard–Chipart Criterion). The polynomial A is stable if and only if one of the following four conditions holds:

(a) $a_n > 0,\ a_{n-2} > 0, \ldots, a_{n-2m} > 0,\ \Delta_1 > 0,\ \Delta_3 > 0, \ldots, \Delta_{2m'-1} > 0$;

(b) $a_n > 0,\ a_{n-2} > 0, \ldots, a_{n-2m} > 0,\ \Delta_2 > 0,\ \Delta_4 > 0, \ldots, \Delta_{2m} > 0$;

(c) $a_n > 0, a_{n-1} > 0, a_{n-3} > 0, \ldots, a_{n-2m'+1} > 0, \Delta_1 > 0, \Delta_3 > 0, \ldots, \Delta_{2m'-1} > 0$;

(d) $a_n > 0, a_{n-1} > 0, a_{n-3} > 0, \ldots, a_{n-2m'+1} > 0, \Delta_2 > 0, \Delta_4 > 0, \ldots, \Delta_{2m} > 0$,

where m and m' are the integer parts of $n/2$ and $(n+1)/2$, respectively, and $\Delta_1, \Delta_2, \ldots, \Delta_n$ are the Hurwitz determinants of A.

4. Brief review of algebraic methods

Problems 1–4 formulated in Section 2 may be solved effectively by using algebraic methods with exact symbolic computation. Such methods may be divided into two categories: one category dealing with systems of polynomial equations and inequations by means of variable elimination and triangular decomposition and the other dealing with systems of polynomial equations and inequalities using real quantifier elimination and solution classification. Here we provide a brief review of some of the well-known algebraic methods.

4.1. Dealing with polynomial systems

Triangular Sets. Let $\mathbf{Q}[\boldsymbol{x}]$ denote the ring of polynomials in $\boldsymbol{x}$ with rational coefficients. A *triangular set* is a finite nonempty set of polynomials of the form

$$[T_1(x_1, \ldots, x_{p_1}), T_2(x_1, \ldots, x_{p_2}), \ldots, T_r(x_1, \ldots, x_{p_r})]$$

with $0 < p_1 < \cdots < p_r \leq n$ and each $T_i \in \mathbf{Q}[\boldsymbol{x}]$ having positive degree in x_{p_i}. In the case where $r = n$, we have $x_{p_i} = x_i$. Effective algorithms have been developed by Wu [49], Lazard [23], Kalkbrener [19], and Wang [40, 41, 42] to decompose any polynomial set $\mathbb{P}$ or system $[\mathbb{P}, \mathbb{Q}]$ (i.e., a pair of polynomial sets, where $\mathbb{P},\ \mathbb{Q} \subset \mathbf{Q}[\boldsymbol{x}]$) into triangular systems $[\mathbb{T}_i, \mathbb{U}_i]$ of various kinds such that

$$\mathrm{Zero}(\mathbb{P}/\mathbb{Q}) = \bigcup_{i=1}^{e} \mathrm{Zero}(\mathbb{T}_i/\mathbb{U}_i), \tag{4.1}$$

where each $\mathbb{T}_i$ is a triangular set and $\mathrm{Zero}(\mathbb{P}/\mathbb{Q})$ denotes the set of all common zeros of the polynomials in $\mathbb{P}$ which are not zeros of any polynomial in $\mathbb{Q}$. The triangular sets $\mathbb{T}_i$ and systems $[\mathbb{T}_i, \mathbb{U}_i]$ may satisfy some additional requirements for being *regular*, *simple*, or *irreducible* [3, 4, 43].

Gröbner Bases. For any finite nonempty set $\mathbb{P}$ of polynomials in $\mathbf{Q}[\boldsymbol{x}]$, Buchberger's algorithm [8] can be used to compute a special set $\mathbb{G}$ of polynomials, called

a *Gröbner basis* of $\mathbb{P}$, such that the ideal generated by the polynomials in $\mathbb{G}$ is the same as that generated by the polynomials in $\mathbb{P}$, while $\mathbb{G}$ is well structured and has many remarkable properties. For example, any Gröbner basis with respect to the *purely lexicographical* (plex) term order is in triangular form.

Plex Gröbner bases are convenient for application in various situations, but their computation is relatively expensive. An efficient strategy is to compute the Gröbner basis first with respect to one admissible term order (under which Gröbner bases are easier to compute) and then convert the computed Gröbner basis into a Gröbner basis with respect to another term order, e.g., using the well-known FGLM algorithm [16]. Besides many improvements to the original algorithm of Buchberger, more efficient algorithms for Gröbner bases computation have been developed, e.g., by Faugère [15].

Resultants. The method of resultants is a classical tool in elimination theory. Resultants are usually constructed as determinants of certain matrices with the coefficients of given polynomials or 0 as entries. They provide a simple and effective way to eliminate one or several variables simultaneously from the given set $\mathbb{P}$ of polynomials, allowing one to triangularize $\mathbb{P}$ or to establish conditions for $\mathbb{P}$ to have zeros. See, e.g., [43, Section 5.4], [14], and references therein for more information about the classical theory and modern developments of resultants.

The methods of triangular sets, Gröbner bases, and resultants may be used to solve systems of polynomial equations over the field of complex numbers, but in general they are not applicable to problems involving inequalities over $\mathbf{R}$.

4.2. Dealing with semi-algebraic systems

Cad. For any given system of polynomial equations and inequalities, the method of cylindrical algebraic decomposition (CAD) proposed by Collins [11] and improved by Hong and others (see, e.g., [12]) may be used to decompose the n-dimensional real space $\mathbf{R}^n$ into finitely many cylindrically arranged regions, called *cells*, such that every polynomial from the given system is sign-invariant in each cell. Since the signs of all the polynomials in each cell of the decomposition can be easily determined by computing the values of the polynomials at a sample point, one is able to eliminate, by computing a CAD, the quantifiers of any quantified formula over real closed fields.

Real Solution Classification. Yang and Xia [52, 51] proposed a practical method for real solution classification of any semi-algebraic system $\mathcal{S}$. The method works by first decomposing the set of equations in $\mathcal{S}$ into regular triangular sets $\mathbb{T}_1, \ldots, \mathbb{T}_e$ and then computing a so-called *border polynomial* B from each $\mathbb{T}_i$ and the inequalities in $\mathcal{S}$ such that the number of distinct real zeros of $\mathbb{T}_i$ is constant in each cell of the complement of $B = 0$ in the space of parameters. The construction of B requires the computation of resultants, discriminants, and generalized discriminant sequences [52]. The conditions on the parameters for any classification of the real solutions of $\mathcal{S}$ may be obtained by applying an improved version of the partial CAD algorithm of Collins and Hong [12] to the border polynomials to

decompose the parameters space into finitely many cells and then computing the number of distinct real solutions of $\mathcal{S}$ at a sample point in each cell.

Discriminant Varieties. A discriminant variety V of a parametric semi-algebraic system

$$p_1(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \ldots, p_s(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \tag{4.2}$$

$$q_1(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \ldots, q_e(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \tag{4.3}$$

introduced by Lazard and Rouillier [24, 25], is a semi-algebraic subset of the real space $\mathbf{R}^m$ of parameters $\boldsymbol{\lambda}$ satisfying the following property: on each connected open subset of $\mathbf{R}^m$ not meeting V, the number of real distinct solutions of (4.2) is constant and the signs of all the q_i at the real solutions of (4.2) are invariant. System (4.2)–(4.3) is *well-behaved* if $s = n$ (the number of variables), all the parameters $\boldsymbol{\lambda}$ are independent, and for almost all parametric values $\bar{\boldsymbol{\lambda}}$ of $\boldsymbol{\lambda}$, the ideal generated by $p_1|_{\boldsymbol{\lambda}=\bar{\boldsymbol{\lambda}}}, \ldots, p_s|_{\boldsymbol{\lambda}=\bar{\boldsymbol{\lambda}}}$ is radical and zero-dimensional. For any well-behaved system, one may compute its minimal discriminant variety, that is the intersection of all its discriminant varieties, by using Gröbner bases. Therefore, the problem of determining the number of real solutions of (4.2)–(4.3) may be reduced to a similar problem depending only on the parameters. The latter can be solved, for example, by using partial CAD and the former is then solved by computing the number of real solutions of (4.2)–(4.3) at sample points.

Popular computer algebra systems such as Maple and Mathematica have built-in functions for the computation of Gröbner bases and resultants. There are special-purpose packages for computing triangular sets, Gröbner bases, multivariate resultants, discriminant varieties, real solving and solution classification, and doing quantifier elimination. The reader may consult [45] for more information about such software tools. In this paper, we use mainly the packages DISCOVERER and DV for our experiments. A short presentation of these two packages will be given in Section 9.1.

5. Stability analysis using algebraic methods

Our general approach for stability analysis of biological systems using algebraic methods works by reducing the problem of stability analysis to the four problems formulated in Section 2. Now we explain how these problems may be solved by using the algebraic methods reviewed in Section 4.

Step 0. Assume that the biological system in question is modeled by the dynamical system (2.1). Form the system (2.2) of polynomial equations and inequations. If the variables $\boldsymbol{x}$ and parameters $\boldsymbol{\lambda}$ have some additional constraints (in view of their physical values; for example, the concentration of a protein cannot be negative), then add such (equality and inequality) constraints to (2.2). Without

loss of generality,[1] let the constraints be given as

$$\begin{aligned} &P_{n+1}(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \ldots, P_s(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \\ &Q_{n+1}(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \ldots, Q_t(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \end{aligned} \tag{5.1}$$

where $s, t \geq n$.

Step 1. By using the method of triangular sets, Gröbner bases, or resultants sketched in Section 4.1, we triangularize the set $\mathbb{P} = \{P_1, \ldots, P_s\}$ of polynomials to obtain one or several triangular sets $\mathbb{T}_k$. These triangular sets may be subject to satisfy certain conditions, depending on which method will be used to deal with the inequality relations. If the parameters $\boldsymbol{\lambda}$ are not present, then go to step 2; otherwise, go to step 3.

Step 2. Isolate the real zeros of each $\mathbb{T}_k$ by rational intervals using existing algorithms, for example, those presented in [34, 50]. In this way, one can obtain all real zeros of $\mathbb{P}$, represented by rational intervals. Then the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at each real zero may be determined by computing the values of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at the ends of the rational intervals, provided that the width of the intervals is sufficiently small as required. Therefore, Problems 1 and 3 are solved.

Step 3. For each triangular set $\mathbb{T}_k$, use the inequality polynomials $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ to compute an algebraic variety V in $\boldsymbol{\lambda}$ that decomposes the real space $\mathbf{R}^m$ of parameters into finitely many cells such that in each cell the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at these real zeros remain invariant. This can be done, for example, by using the method of CAD, real solution classification, or discriminant varieties explained in Section 4.2. Then one takes a sample rational point from each cell, isolate the real zeros of $\mathbb{T}_k$ by rational intervals, and compute the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at this sample point. In this way, the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at these real zeros in each cell are determined.

Step 4. Meanwhile, one may obtain the signs of (the factors of) the defining polynomials of V at each sample point. If the conditions on $\boldsymbol{\lambda}$ for system (2.1) to have a prescribed number of real zeros are desired, we form the conditions according to the signs at the sample points of those cells in which the system has exactly the prescribed number of real zeros. By now Problems 2 and 4 are completely solved.

In [46], Wang and Xia have shown in detail how to use regular triangular sets with additional requirements (computed by the algorithms described in [42, 53] and the Epsilon function RegSer [44]) and the method of real solution classification presented in [51, 52] according to the above approach. Several examples are given

[1] Note that $F \neq 0$, $F \geq 0$, and $F \leq 0$ may be written as $F > 0$ or $-F > 0$, $F = 0$ or $F > 0$, and $F = 0$ or $-F > 0$ respectively, so constraints involving inequalities of other forms can be reduced to the form (5.1).

in [46, 47]. In these papers, the variety V is defined by a border polynomial of system (2.2). In Section 7, we will see that CAD and discriminant varieties may be used instead of real solution classification.

As remarked in Section 2, for bifurcation points [21] where the real parts of some eigenvalues of $\boldsymbol{J}$ are 0 and the technique of linearlization may not work, the analysis of stability becomes difficult. So one may exclude this case by including the bifurcation conditions in (5.1). The conditions to rule out the fold bifurcation and Hopf bifurcation are

$$\det(\boldsymbol{J}) \neq 0 \quad \text{and} \quad \det(\boldsymbol{J}) \leq 0 \ \text{ or } \ \det(2\boldsymbol{J} \odot \boldsymbol{I}) \neq 0, \tag{5.2}$$

respectively, where $2\boldsymbol{J} \odot \boldsymbol{I}$ is the *bialternate product* of $2\boldsymbol{J}$ and $\boldsymbol{I}$ defined below. For two $n \times n$ matrices $\boldsymbol{A} = (a_{ij})$ and $\boldsymbol{B} = (b_{ij})$ with

$$m = \frac{n(n-1)}{2},$$

the bialternate product $\boldsymbol{A} \odot \boldsymbol{B}$ is an $m \times m$ matrix $\boldsymbol{C} = (c_{i,j})$, whose elements are given by

$$c_{\frac{(p-1)(p-2)}{2}+q,\frac{(r-1)(r-2)}{2}+s} = \frac{1}{2}\left\{\begin{vmatrix} a_{pr} & a_{ps} \\ b_{qr} & b_{qs} \end{vmatrix} + \begin{vmatrix} b_{pr} & b_{ps} \\ a_{qr} & a_{qs} \end{vmatrix}\right\}$$

for $p, r = 2, \ldots, n$, $q = 1, \ldots, p-1$, and $s = 1, \ldots, r-1$.

Under the conditions (5.2), the real parts of the eigenvalues of $\boldsymbol{J}$ are nonzero. Observe that, if the real part of an eigenvalue changes its sign from negative to positive (or vice verse) as the parametric values change, it must pass through 0. Therefore, if the bifurcation conditions are included in (5.1), then in each cell of the real space $\mathbf{R}^m$ of parameters decomposed by the algebraic variety in $\boldsymbol{\lambda}$ computed using the inequality polynomials $Q_1, \ldots, Q_t$ only, the signs of $H_1, \ldots, H_r$ at the real zeros of $\mathbb{T}_k$ are also invariant. It follows that the signs of $H_1, \ldots, H_r$ at the real zeros of $\mathbb{T}_k$ in each cell may be determined simply by computing their values at a sample point. This allows us to modify step 3 above as follows.

Step 3′. Assume that the bifurcation conditions (5.2) are included in (5.1). For each triangular set $\mathbb{T}_k$, use the inequality polynomials $Q_1, \ldots, Q_t$ to compute an algebraic variety V in $\boldsymbol{\lambda}$ that decomposes the real space $\mathbf{R}^m$ of parameters into finitely many cells such that in each cell the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ at these real zeros remain invariant. Then take a sample rational point from each cell, isolate the real zeros of $\mathbb{T}_k$ by rational intervals, and compute the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at this sample point. In this way, the number of real zeros of $\mathbb{T}_k$ and the signs of $Q_1, \ldots, Q_t$ and $H_1, \ldots, H_r$ at these real zeros in each cell are determined.

As the usually large polynomials $H_1, \ldots, H_r$ are not used, the computation of the variety V and thus the cell decomposition become easier. Determining the signs of $H_1, \ldots, H_r$ in each cell is relatively inexpensive. The use of the bifurcation conditions to improve the approach was proposed first by Chen [9].

6. Bifurcation analysis and limit cycles

Stability analysis based on the technique of linearization presented in Section 2 may fail at bifurcation points because near such points the behavior of system (2.1) may differ qualitatively from that of its linearized system (see [21, 28, 54]). In this section, we consider the case of Hopf bifurcation for $n = 2$ and $Q_1, Q_2 \in \mathbf{Q}[\boldsymbol{\lambda}]$, a difficult case where limit cycles may bifurcate. In this case, the characteristic polynomial of the Jacobian matrix $\boldsymbol{J}$ has a pair of purely imaginary roots and the differential system is said to be of *center-focus type*. The study of limit cycles in this case is a subject of active research. It is closely related to Hilbert's 16th problem [39, 54].

Let $n = 2$, $\bar{\boldsymbol{x}} = (\bar{x}_1, \bar{x}_2)$ be a steady state of system (2.1), and $\bar{\boldsymbol{J}}_2$ be the Jacobian matrix

$$\begin{bmatrix} a(\boldsymbol{\lambda}, \boldsymbol{x}) & b(\boldsymbol{\lambda}, \boldsymbol{x}) \\ c(\boldsymbol{\lambda}, \boldsymbol{x}) & d(\boldsymbol{\lambda}, \boldsymbol{x}) \end{bmatrix}$$

of (2.1) at $\bar{\boldsymbol{x}}$. Then the characteristic polynomial of $\bar{\boldsymbol{J}}_2$ has a pair of purely imaginary roots only if $\bar{\boldsymbol{x}}$ satisfies the conditions

$$a + d = 0, \quad -a^2 - bc > 0. \tag{6.1}$$

The problems of deciding whether the steady states of system (2.1) without parameters satisfy the conditions (6.1) and determining the conditions on the parameters $\bar{\boldsymbol{\lambda}}$ (when they are present) for the steady states of (2.1) to satisfy (6.1) may be reduced to Problems 3 and 4, and thus may be solved by using the method explained in Section 5.

Now assume that the conditions (6.1) are satisfied. We want to analyze the stability and bifurcation of limit cycles of system (2.1) with $n = 2$ in this case. To this end, we make a linear transformation

$$x_1 = -\frac{1}{c} y_2 - \frac{a}{c\delta} y_1 + \bar{x}_1, \quad x_2 = -\frac{1}{\delta} y_1 + \bar{x}_2, \quad t = \frac{\tau}{\delta},$$

where $\delta = \sqrt{-a^2 - bc}$. Then system (2.1) with $n = 2$ is transformed into the following canonical form

$$\begin{aligned} \frac{\mathrm{d}y_1}{\mathrm{d}\tau} &= y_2 + F_1(\boldsymbol{\lambda}, \delta, \bar{x}_1, \bar{x}_2, y_1, y_2), \\ \frac{\mathrm{d}y_2}{\mathrm{d}\tau} &= -y_1 + F_2(\boldsymbol{\lambda}, \delta, \bar{x}_1, \bar{x}_2, y_1, y_2), \end{aligned} \tag{6.2}$$

where F_1 and F_2 are polynomials in $\mathbf{Q}(\boldsymbol{\lambda}, \delta)[\bar{x}_1, \bar{x}_2, y_1, y_2]$.

Then using the method described in [39] we can compute so-called *Liapunov constants* (or *focal values*) $v_3, v_5, \ldots, v_{2j+1}, \ldots$ in $\boldsymbol{\lambda}, \delta, \bar{x}_1, \bar{x}_2$ such that the differential of a locally positive polynomial $L(y_1, y_2) \in \mathbf{Q}(\boldsymbol{\lambda}, \delta)[\bar{x}_1, \bar{x}_2, y_1, y_2]$ along the integral curve of (6.2) is of the form

$$\frac{\mathrm{d}L(y_1, y_2)}{\mathrm{d}\tau} = v_3 y_2^4 + v_5 y_2^6 + \cdots + v_{2j+1} y_2^{2j+2} + \cdots .$$

According to Liapunov's theorem [28, 54], the stability of the steady state $(0, 0)$ for system (6.2) and thus $\bar{\boldsymbol{x}}$ for system (2.1) is determined by the sign of $\mathrm{d}L/\mathrm{d}\tau$ and therefore by the sign of the first nonzero Liapunov constant v_{2k+1}. Namely, we have the following simple criteria.

Theorem 4 ([39, 54]). For any given parametric values $\bar{\boldsymbol{\lambda}}$ of $\boldsymbol{\lambda}$ and steady state $\bar{\boldsymbol{x}}$ of system (2.1) with $n = 2$ and $Q_1, Q_2 \in \mathbf{Q}[\boldsymbol{\lambda}]$,

(a) if there is an integer $k \geq 1$ such that $v_3 = \cdots = v_{2k-1} = 0$ but $v_{2k+1} \neq 0$, then $\bar{\boldsymbol{x}}$ is unstable when $v_{2k+1} > 0$, and asymptotically stable when $v_{2k+1} < 0$;
(b) if $v_{2j+1} = 0$ for all $j = 1, 2, \ldots$, then $\bar{\boldsymbol{x}}$ is stable of *center type*, but not asymptotically stable.

In case (a), the steady state $\bar{\boldsymbol{x}}$ of system (2.1) is said to be a *focus of order* k. In case (b), $\bar{\boldsymbol{x}}$ is said to be a *center* of (2.1). By Theorem 4 (a), the problem of determining the stability of a focus is reduced to that of determining the signs of the Liapunov constants and thus again reduced to Problems 3 and 4. Recall that in this case the parameters $\boldsymbol{\lambda}$, the steady state $\boldsymbol{x} = \bar{\boldsymbol{x}}$, and the introduced variable δ satisfy the constraints (2.2) and (5.1) with $n = 2$, (6.1), and $\delta^2 + a^2 + bc = 0$. Under these constraints, system (2.1) is of center-focus type.

When the steady state $\bar{\boldsymbol{x}}$ of (2.1) is a focus of order k, one may construct k small-amplitude limit cycles near $\bar{\boldsymbol{x}}$ by small perturbation. We will show how these limit cycles may be constructed for concrete biological systems in a forthcoming paper.

Determining conditions for $\bar{\boldsymbol{x}}$ to be a center from the computed Liapunov constants is a tougher issue because the conditions in Theorem 4 (b) are given by infinitely many equalities (in a finite number of variables). By means of Liapunov constants, one can (only) establish the necessary conditions for $\bar{\boldsymbol{x}}$ to be a center. To derive the conditions, one needs to decide whether or under which conditions on the parameters $\boldsymbol{\lambda}$ the Liapunov constants $v_3, v_5, \ldots$ are 0. This may again be reduced to Problems 3 and 4 and thus may be tackled by using algebraic methods (as shown in the extensive literature on the derivation of center conditions). However, proving the sufficiency of the established necessary conditions requires more sophisticated mathematical techniques and algebraic computations. The main difficulty in deriving center conditions and searching for differential systems having high-order foci from Liapunov constants comes from the large polynomials that cannot be effectively managed even on a powerful computer.

7. Application of CAD and discriminant varieties

In this section, we explain how to apply the methods of CAD and discriminant varieties to stability analysis of biological systems and illustrate some of the computational steps by an example. This application was investigated initially by the first author in [30].

7.1. Stability analysis using the CAD method

The CAD method is designed mainly for quantifier elimination (QE). To use this method, one needs to formulate the problem of stability analysis as a quantified formula. Then the CAD method may be applied to decide whether the formula is true or false, or to obtain an equivalent quantifier-free formula. The problem of existence of real solutions of the semi-algebraic system (2.2) + (5.1) may be easily formulated as a quantified formula, so it can be solved by simple application of the CAD method. Therefore, CAD and other QE methods may be directly applied to determine the conditions on $\boldsymbol{\lambda}$ for (2.2) + (5.1) to have real solutions. However, it is not straightforward to formulate other problems of stability analysis (such as determining the conditions for (2.2) + (5.1) to have exactly the prescribed number of distinct real solutions) as quantified formulas. The formulation of quantified formulas is possible in some circumstances with additional tricks, but the obtained formulas may become complicated. It turns out that QE methods are not very suitable for dealing with stability problems about the *number* of (stable) steady states.

However, the QEPCAD package [6] (that implements an improved QE method by partial CAD) provides the functionality of determining the conditions for a single polynomial to have at least k real roots (for any given integer $k \geq 0$). This functionality allows us to determine the number of (stable) steady states in various situations. In fact, we have analyzed the stability of several biological systems using QEPCAD.

7.2. Stability analysis using discriminant varieties

The method of discriminant varieties (DV), described in [25] and reviewed very briefly in Section 4, provides a good alternative for stability analysis. It can be used to compute the minimal discriminant variety V of the semi-algebraic system composed of (2.2), (5.1), and $H_1 > 0, \ldots, H_r > 0$. The variety V decomposes the real space $\mathbf{R}^m$ of parameters into cells, such that in each cell (not on V) the number of real solutions of the system is constant and the sign of each H_i at the real solutions is invariant. The signs of the H_i can be determined by computing the values of H_i at sample points. It follows that the DV method can be directly applied to the four problems formulated in Section 2.

Although the methods of DV [25] and real solution classification [51, 52] are similar, they have some major differences: Gröbner bases are used as the main computational engine in the DV method for the triangularization of the polynomial equations from the semi-algebraic system, while in the method of real solution classification the algorithms of Wu [49] and Wang [42] are used to compute regular triangular sets. In their implementations, different algorithms are used for isolating the real solutions of triangular sets.

7.3. Illustrative example

In this subsection, we present some of the computational steps for a concrete biological system to illustrate how the methods of CAD and discriminant varieties

may be applied. The system is a simple model of the antagonistic interactions between cyclin-dependent kinases and the anaphase promoting complex [37]. The model can be described by a pair of nonlinear ordinary differential equations

$$\begin{aligned} \frac{\mathrm{d}x}{\mathrm{d}t} &= k_1 - (k_2' + k_2'' y)x, \\ \frac{\mathrm{d}y}{\mathrm{d}t} &= \frac{(k_3' + k_3'' A)(1-y)}{J_3 + 1 - y} - \frac{k_4 m x y}{J_4 + y}, \end{aligned} \tag{7.1}$$

where x and y are the average concentrations (grams of protein per gram of total cell mass) of cyclin B/Cdk dimers and active Cdh1/APC complexes respectively, $k_1, k_2', k_2'', k_3', k_3'', k_4$ are rate constants, J_3, J_4 are Michaelis constants, and m is a real parameter representing cell "mass". Considering the physical background of the biological problem, we assume that $x > 0$, $y > 0$, $m > 0$. By taking numerical values for the biological constants

$$\begin{aligned} &k_1 = \frac{1}{25}, \quad k_2' = \frac{1}{25}, \quad k_2'' = 1, \quad k_3' = 1, \quad k_3'' = 10, \quad k_4 = 35, \\ &J_3 = \frac{1}{25}, \quad J_4 = \frac{1}{25}, \quad A = 0 \end{aligned}$$

as in [37], we obtain from (7.1) the following semi-algebraic system

$$\begin{aligned} &1 - (1 + 25\,y)x = 0, \\ &(1-y)(1+25\,y) - 35\,mxy(26 - 25\,y) = 0, \\ &26 - 25\,y \neq 0,\ x > 0,\ y > 0,\ m > 0. \end{aligned} \tag{7.2}$$

Consider moreover the Hurwitz determinants

$$\begin{aligned} H_1 &= -23 + 75\,y + 910\,mx - 1750\,mxy, \\ H_2 &= -24 - 550\,y + 910\,mx - 1750\,mxy + 1250\,y^2 - 21875\,mxy^2. \end{aligned}$$

First, we show how to employ the CAD method to analyze the stability of system (7.1) by using the package QEPCAD [6]. For any polynomial Φ in `x` and integer `n` > 0, the QEPCAD command

```
(E x) [ x = _ root _ n Φ ]
```

means that Φ has at least `n` distinct real roots for `x`.

Using the first equation to eliminate x from the second equation in (7.2), we obtain

$$P = -625\,y^3 + 575\,y^2 + 875\,my^2 + 49\,y - 910\,my + 1.$$

Then by means of the QEPCAD command above, we may determine the conditions for system (7.2) to have any prescribed number of real solutions: (7.2) has one real solution if $R < 0$, two real solutions if $R = 0$, and three real solutions if $R > 0$, where

$$R = 28983500\,m^3 - 51273600\,m^2 + 22577975\,m - 1898208; \tag{7.3}$$

system (7.2) cannot have four or more real solutions.

Now we proceed to determine how many of the steady states are stable or not. For example, under the assumption $R = 0 \wedge m > 0$, system (7.2) has two real solutions. We can examine whether or not the two steady states of (7.1) are both stable in this case by formulating the problem as

$$(\forall\, y)\, [\, P = 0 \Rightarrow (H_1 > 0 \wedge H_2 > 0)\,].$$

The false of this formula may be determined by QEPCAD, meaning that not both of the steady states are stable. Note that the assumption is imposed by the QEPCAD command `assume`. Then we can use the formula

$$(\exists\, y)\, [\, P = 0 \wedge H_1 > 0 \wedge H_2 > 0\,]$$

(for which the QEPCAD output is `true`) to confirm that one of the two steady states is stable.

Similarly, under the assumption $R > 0 \wedge m > 0$, we can examine whether the three steady states of system (7.1) are stable or not by using the following formulas:

$$\begin{aligned}
&(\forall\, y)\, [\, P = 0 \Rightarrow (H_1 > 0 \wedge H_2 > 0)\,] && \text{(output: } \texttt{false}\text{)},\\
&(\exists\, y_1)\,(\exists\, y_2)\, [\, y_1 \neq y_2 \wedge P|_{y=y_1} = 0 \wedge P|_{y=y_2} = 0 \wedge H_1|_{y=y_1} > 0 \wedge H_1|_{y=y_2} > 0\\
&\qquad\qquad \wedge H_2|_{y=y_1} > 0 \wedge H_2|_{y=y_2} > 0\,] && \text{(output: } \texttt{true}\text{)},\\
&(\exists\, y)\, [\, P = 0 \wedge (H_1 < 0 \vee H_2 < 0)\,] && \text{(output: } \texttt{true}\text{)}.
\end{aligned}$$

Under the assumption $R < 0 \wedge m > 0$, we can examine whether the only steady state of system (7.1) is stable or not by using the formula

$$(\forall y)\, [\, P = 0 \Rightarrow (H_1 > 0 \wedge H_2 > 0)\,] \qquad \text{(output: } \texttt{true}\text{)}.$$

Therefore, the following results are obtained:

- if $R < 0$, then system (7.1) has one stable steady state;
- if $R = 0$, then system (7.1) has two steady states, of which one is stable;
- if $R > 0$, then system (7.1) has three steady states, of which two are stable and the other is unstable.

Note that the method may be used not only to determine the number of stable or unstable steady states as above, but also to identify which of the steady states are stable or unstable. Exact results of this type cannot be established by purely numerical computation.

Next, we present some computational details to illustrate the use of discriminant varieties for stability analysis. We first compute a minimal discriminant variety V in m, defined by

$$\begin{aligned}
W = {} & [\, 28983500\, m^3 - 51273600\, m^2 + 22577975\, m - 1898208 = 0\,] \vee\\
& [\, 28983500\, m^3 - 51273600\, m^2 + 5944995\, m - 949104 = 0\,] \vee [\, m = 0\,],
\end{aligned}$$

of the semi-algebraic system composed of (7.2) and $H_1 > 0, H_2 > 0$. Then we need to determine the number of (stable) steady states in each cell. Using any available

algorithm, we can isolate all the four positive real roots m_i of the polynomials in W:

$$m_1 \in \left[\frac{14235354009}{8589934592}, \frac{14235354011}{8589934592}\right], \quad m_2 \in \left[\frac{1884871821}{17179869184}, \frac{7539487289}{68719476736}\right],$$
$$m_3 \in \left[\frac{2264819159}{4294967296}, \frac{283102395}{536870912}\right], \qquad m_4 \in \left[\frac{4862025063}{4294967296}, \frac{607753133}{536870912}\right].$$

These four real roots divide the half line $m > 0$ into five intervals. We choose one sample point in each interval and isolate the real solutions of system (7.2) for x, y at the same point. Finally, we compute the signs of the Hurwitz determinants at each sample point to determine the stability of the steady states.

For the cases in which $m = m_i$ $(1 \le i \le 4)$, we can add the corresponding polynomial equation in W to system (7.2) and then isolate the real solutions of the new system (which has no parameter).

In summary, we have the conditions shown in Table 1 on the parameter m for system (7.1) to have prescribed numbers of (stable) steady states. These conditions are consistent with the results established by using the CAD method.

TABLE 1. Numbers of (stable) steady states of (7.1)

m	$0<m<\mu_1$	$m=\mu_1$	$\mu_1<m<\mu_2$	$m=\mu_2$	$\mu_2<m<\mu_3$	$m=\mu_3$	$\mu_3<m<+\infty$
Steady states	1	2	3	2	1	2	3
Stable states	1	*	2	*	1	*	2

where

$$\mu_1 \approx 0.1097139798, \quad \mu_2 \approx 0.5273193027, \quad \mu_3 \approx 1.132028425$$

are the three real roots of R in (7.3). In the cases where $m = \mu_1$, μ_2, or μ_3 marked with *, one of the two steady states is stable and the other makes $H_1 = 0$ or $H_2 = 0$ (which means that the linearization method is inapplicable in these cases).

8. Stability and bifurcation analysis of self-assembling micelle systems with chemical sinks

Consider the following dissipative dynamical system studied in [5]:

$$\begin{aligned} \frac{\mathrm{d}x}{\mathrm{d}\tau} &= \mu - xy^2 - x(r+\alpha) = p, \\ \frac{\mathrm{d}y}{\mathrm{d}\tau} &= rx + xy^2 - \eta y = q. \end{aligned} \tag{8.1}$$

In [47] stability conditions on the parameters are obtained for the system in the special case where $\alpha = \eta = f$. For this more general system, we are able to establish

the stability conditions using the algorithmic approach presented in Section 5. The conditions obtained by means of the DV method are summarized as follows. Let

$$\begin{aligned} R_1 &= 4\,(r+\alpha)^3\eta^4 + (8\,r^2 - 20\,r\alpha - \alpha^2)\mu^2\eta^2 + 4\,r\mu^4, \\ R_2 &= r(r\alpha + 4\,r + 4\,\alpha)\eta^5 + (r+\alpha)(2\,r+\alpha)^2\eta^4 - 4\,r\mu^2\eta^3 \\ &\quad + (8\,r^2 - 8\,r\alpha - \alpha^2)\mu^2\eta^2 - 8\,r\alpha^2\mu^2\eta + 4\,r\mu^4 + \alpha^4\mu^2. \end{aligned}$$

Then:

- if $R_1 < 0$ and $R_2 \neq 0$, then system (8.1) has three steady states, of which two are stable;
- if $R_1 > 0$ and $R_2 > 0$, then system (8.1) has only one stable steady state;
- if $R_1 > 0$ and $R_2 < 0$, then system (8.1) has only one unstable steady state;
- if $R_1 = 0$ and $\alpha - 8\,r \neq 0$, then system (8.1) has two steady states, of which one is stable;
- if $R_1 = 0$ and $\alpha - 8\,r = 0$, then system (8.1) has one unstable steady state.

The conditions in the case $R_2 = 0$ are very complicated and we do not produce them here.

Now we want to derive conditions for system (8.1) to be of center-focus type, as well as center conditions and stability conditions of its foci. For this purpose, let us first compute the plex Gröbner basis of $\{p, q\}$ with $y \prec x$: the basis consists of two polynomials

$$g_1 = \eta y^3 - \mu y^2 + r\eta y + \alpha\eta y - r\mu, \quad g_2 = \alpha x + \eta y - \mu.$$

The system $g_1 = 0$, $g_2 = 0$ has real solutions for any parametric values of μ, r, and $\alpha \neq 0, \eta \neq 0$. Therefore, for $\alpha\eta \neq 0$ system (8.1) always has steady states. Let $\alpha\eta \neq 0$ and $y_0 = w$ be a real root of g_1. Then

$$x_0 = \frac{\mu - \eta w}{\alpha}$$

is a real root of g_2. The Jacobian matrix of (8.1) at (x_0, y_0) is

$$\begin{bmatrix} a & b \\ c & d \end{bmatrix} = \begin{bmatrix} -(w^2 + r + \alpha) & -\frac{2\,w(\mu - \eta w)}{\alpha} \\ r + w^2 & \frac{2\,w(\mu - \eta w)}{\alpha} - \eta \end{bmatrix}.$$

System (8.1) becomes of center-focus type if

$$\begin{aligned} f_1 &= \alpha w^2 + 2\,\eta w^2 - 2\,\mu w + r\alpha + \alpha\eta + \alpha^2 = -(a+d)\alpha = 0, \\ f &= (\alpha - \eta)w^2 - r\eta + r\alpha + \alpha^2 = a^2 + bc + (a+d)(r + w^2) < 0. \end{aligned}$$

Note that

$$f_2 = g_1|_{y=w} = \eta w^3 - \mu w^2 + r\eta w + \alpha\eta w - r\mu = 0.$$

From $f_1 = f_2 = 0$, $f < 0$ and by using DISCOVERER or DV (see Section 9.1), one can obtain conditions, say (CF), in the parameters η, μ, r, α for (8.1) to be of center-focus type, under which limit cycles may bifurcate from (x_0, y_0). The conditions (CF) are quite complicated and we do not produce them here. It may also be proved easily (e.g., by using DISCOVERER) that, if $\eta = \alpha$, then there are no real values of $\mu, r, \alpha \neq 0$ that satisfy (CF). This confirms the conclusion in [5]

that Hopf bifurcations are absent and (CF) hold only for non-physical values of α in this case. However, there do exist real values of μ, r, η, α such that $0 \neq \eta \neq \alpha \neq 0$ and (CF) hold, as we will see clearly below.

Under the conditions (CF), we make a linear transformation

$$x = -\frac{1}{c}Y - \frac{a}{c\delta}X + x_0, \quad y = -\frac{1}{\delta}X + y_0, \quad \tau = \frac{t}{\delta},$$

where $\delta = \sqrt{-f}$. Then system (8.1) is transformed into the following canonical form

$$\begin{aligned} \frac{\mathrm{d}\,X}{\mathrm{d}\,t} &= Y + \frac{\delta}{\alpha}Q, \\ \frac{\mathrm{d}\,Y}{\mathrm{d}\,t} &= -X + Q, \end{aligned} \tag{8.2}$$

where

$$\begin{aligned} Q &= \frac{\gamma}{(r+w^2)\delta^3}X^2 - \frac{2\,\alpha w}{(r+w^2)\delta^2}XY - \frac{\alpha(r+\alpha+w^2)}{(r+w^2)\delta^4}X^3 + \frac{\alpha}{(r+w^2)\delta^3}X^2Y, \\ \gamma &= 2\,\alpha w^3 + \eta w^3 - \mu w^2 + 2\,\alpha^2 w + 2\,r\alpha w + r\eta w - r\mu. \end{aligned}$$

The Liapunov constants of (8.2) may be computed by the function `miscel[licon]` in the Epsilon library [44]. The first Liapunov constant is

$$\begin{aligned} v_3 = {} & \frac{\alpha}{3\,(r+w^2)\delta^3} - \frac{r+\alpha+w^2}{(r+w^2)\delta^3} - \frac{2\,\alpha w\gamma}{3\,(r+w^2)^2\delta^5} + \frac{2\,w\gamma}{3\,\alpha(r+w^2)^2\delta^3} \\ & + \frac{2\,\gamma^2}{3\,\alpha(r+w^2)^2\delta^5}, \end{aligned}$$

whose numerator $\bar{v}_3$, when expanded, has 34 terms. The numerators of the subsequent 5 Liapunov constants $v_5, \ldots, v_{13}$ consist of $384, 1969, 6616, 17504, 39467$ terms and are of total degrees $18, 28, 38, 48, 58$ in the variables $\eta, \mu, r, \alpha, w, \delta$, respectively.

As an illustration of the method and to simplify calculations, let us take $\eta = 1$ and $\mu = 7/10$ as in [5]. We want to determine real values of r and α such that $a + d = 0, a^2 + bc < 0$ and $v_3 = 0$. For this purpose, we compute the plex Gröbner basis $\mathbb{G}$ of $\{f_1, f_2, \delta^2 + f, \bar{v}_3\}$ under the variable ordering $r \prec \alpha \prec w \prec \delta$ using the `Groebner` package in Maple. It is found that the first polynomial in $\mathbb{G}$ may be factorized as $r^2\alpha h$, where

$$\begin{aligned} h = {} & 6146560000\,r^7 - 18562611200\,r^6 + 60883545856\,r^5 - 85487372544\,r^4 \\ & + 55821677296\,r^3 - 16995604984\,r^2 + 2256654801\,r - 61985000. \end{aligned}$$

The polynomial h has only one real root

$$\bar{r} \approx 0.03624946689.$$

The plex Gröbner basis $\mathbb{G}^*$ of $\mathbb{G} \cup \{h, z\alpha - 1\}$ (where z is a new indeterminate) with $r \prec \alpha \prec w \prec \delta \prec z$ is of the form

$$\left[h, l_2\alpha + \sum_{i=0}^{6} l_{2i}r^i, l_3 w + \sum_{i=0}^{6} l_{3i}r^i, l_4\delta^2 + \sum_{i=0}^{6} l_{4i}r^i, l_5 z + \sum_{i=0}^{6} l_{5i}r^i\right],$$

where l_i, l_{ij} are integers of digits between 21 and 28. Let

$$\bar{\alpha} = -\frac{l_{26}\bar{r}^6 + \cdots + l_{21}\bar{r} + l_{20}}{l_2} \approx 0.177105322880358,$$
$$\bar{w} = -\frac{l_{36}\bar{r}^6 + \cdots + l_{31}\bar{r} + l_{30}}{l_3} \approx 0.25315409005153578.$$

It may be easily verified by using the Maple package RS (http://fgbrs.lip6.fr/~rouillie/Software/RS/) that the real zero $(\bar{r}, \bar{\alpha}, \bar{w})$ satisfies $f < 0$, and that for $\eta = 1$, $\mu = 7/10$ and $(r, \alpha) = (\bar{r}, \bar{\alpha})$, $v_3 = 0$ and $v_5 < 0$. Therefore, the steady state $(x_0, y_0) \approx (2.523051835377794, 0.25315409005153578)$ is an asymptotically stable focus of order 2 and thus two limit cycles may bifurcate from (x_0, y_0) for system (8.1) with small perturbation. Detailed construction of these limit cycles will be described in a forthcoming paper.

The results derived in this section demonstrate that algebraic methods can be used effectively to analyze the stability and bifurcations of nontrivial biological systems.

9. Experiments and comparisons

In this section, we report some of our experiments with comparisons for the proposed approaches of stability analysis, provide timing statistics in table form for 15 biological models, and discuss the advantages and disadvantages of different approaches.

9.1. Software tools used

DISCOVERER. The Maple package DISCOVERER, developed by B. Xia, implements the method of Yang and Xia [52, 51] for real solution classification. The main functions `tofind` and `Tofind` of DISCOVERER together with calling sequence take the following form:

`tofind`($[p_1, \ldots, p_s]$, $[q_1, \ldots, q_{r_1}]$, $[q_{r_1+1}, \ldots, q_{r_2}]$, $[g_1, \ldots, g_t]$, $[\boldsymbol{x}]$, $[\boldsymbol{\lambda}]$, N);
`Tofind`($[p_1, \ldots, p_s, R]$, $[q_1, \ldots, q_{r_1}]$, $[q_{r_1+1}, \ldots, q_{r_2}]$, $[g_1, \ldots, g_t]$, $[\boldsymbol{x}]$, $[\boldsymbol{\lambda}]$, N);

corresponding to the semi-algebraic system

$$\begin{cases} p_1(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \ldots, p_s(\boldsymbol{\lambda}, \boldsymbol{x}) = 0, \\ q_1(\boldsymbol{\lambda}, \boldsymbol{x}) \geq 0, \ldots, q_{r_1}(\boldsymbol{\lambda}, \boldsymbol{x}) \geq 0, \\ q_{r_1+1}(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \ldots, q_{r_2}(\boldsymbol{\lambda}, \boldsymbol{x}) > 0, \\ g_1(\boldsymbol{\lambda}, \boldsymbol{x}) \neq 0, \ldots, g_t(\boldsymbol{\lambda}, \boldsymbol{x}) \neq 0, \end{cases} \tag{9.1}$$

where R is a polynomial obtained by `tofind` (see below) and N may take a nonnegative integer or a range. They compute the conditions on the parameters $\boldsymbol{\lambda}$ for system (9.1) to have exactly k distinct real solutions if N is a nonnegative integer k, or k, $k+1$, ..., or l distinct real solutions if N is an integer range $k..l$, or at least k distinct real solutions if N is an indefinite range $k..n$ with n a symbol.

The function `tofind` is called first to find a necessary and sufficient condition, provided that the border polynomial B is not equal to 0. To deal with the case when the parameters are on the boundary, i.e., $B = 0$, one may call `Tofind`, for each factor R of B, to get further results.

DV. The Maple package DV developed by Moroz and Rouillier [29], with main function

$$\texttt{DV\_solve}([p_1, \ldots, p_s], [q_1, \ldots, q_e], [\boldsymbol{\lambda}], [\boldsymbol{x}], \texttt{options});$$

can be used to compute a discriminant variety V from an input semi-algebraic system of the form (4.2)–(4.3), where $\boldsymbol{\lambda}$ is the sequence of parameters and $\boldsymbol{x}$ the sequence of variables in the system. For our stability problem, the polynomials $Q_1, \ldots, Q_t$ from (2.2) and (5.1) and $H_1, \ldots, H_r$ (or bifurcation conditions) as in Section 5 are taken as the inequality polynomials $q_1, \ldots, q_e$ ($e = t + r$) and the number of steady states and the number of stable steady states of the system are constant in each cell of $\mathbf{R}^m$ decomposed by V.

We use DV together with RS for real solving to deal with the problem of stability analysis for biological systems involving only one parameter. For systems involving more parameters, we use the implementation of a partial CAD algorithm contained in DISCOVERER to decompose the real space of parameters into a finite number of cells. In order to automate the process of stability analysis, we have implemented a function `stana` in Maple to interact with the packages DV and RS (as well as the partial CAD implementation) and to determine the signs of the Hurwitz determinants. Taking the background of biological problems into account, our function `stana` is designed for the case in which the parameters are positive and the variables are nonnegative. The function has the following syntax

$$\texttt{stana}([p_1, \ldots, p_s], [q_1, \ldots, q_n], [q_{n+1}, \ldots, q_t], [h_1, \ldots, h_r], [\boldsymbol{\lambda}], [\boldsymbol{x}]),$$

where $p_1, \ldots, p_s$, $\boldsymbol{\lambda}$, and $\boldsymbol{x}$ are as above, $h_1, \ldots, h_r$ are the polynomials $H_1, \ldots, H_r$ whose signs need be determined, and q_i corresponds to the inequality constraint Q_i in (2.2) and (5.1) for $1 \leq i \leq t$. By calling this function, we may obtain a discriminant variety, a list of sample points, the number of steady states, and the number of stable steady states in each cell decomposed by the discriminant variety.

For the biological system studied in Section 7.3, the Maple input to `stana` is as follows:

```
p1:=4-(4+100*y)*x:
p2:=(1-y)*(4+100*y)-35*m*x*y*(104-100*y):
H1:=300*y-92-7000*m*x*y+3640*m*x:
H2:=20000*y^2-8800*y-350000*m*x*y^2-28000*m*x*y-384+14560*m*x:
stana([p1, p2],[],[],[H1, H2],[m],[x,y]);
```

The following output may be returned in less than one second.

$$[\,28983500\,m^3 - 51273600\,m^2 + 5944995\,m - 949104,$$
$$28983500\,m^3 - 51273600\,m^2 + 22577975\,m - 1898208,$$
$$m, 28983500\,m^3 - 51273600\,m^2 + 22577975\,m - 1898208\,]$$

The time of computing DV is:
0.280

The approximate real roots of DV are:
$[\,0.1097139798, 0.5273193027, 1.132028425, 1.657213318\,]$

The numbers of steady states are:
$[\,1, 3, 1, 3, 3\,]$

The numbers of stable steady states are:
$[\,1, 2, 1, 2, 2\,]$

The total time is:
0.484

The list of the numbers of (stable) steady states corresponds to the list of intervals divided by the real roots of the discriminant variety.

9.2. Comparisons and discussions

We have analyzed the stability of a number of biological systems by using Lazard–Rouillier's method of discriminant varieties. For some of these systems the stability has also been analyzed successfully by using Yang–Xia's method of real solution classification according to [46]. In this subsection, we present timing statistics in table form to show the performance of the two methods and discuss their advantages and disadvantages.

To compare the two methods, we have carried out experiments using Xia's DISCOVERER package and our function `stana` (to call DV and RS) for 13 biological models, according to the general approach (with step 3, but not step 3′) described in Section 5. For simplicity of comparison, we use only the main function `tofind` of DISCOVERER without any additional technique and do not consider the cases when the parameters are on the boundaries. The times of computation using the methods of Yang–Xia and Lazard–Rouillier are given in Table 2, where Model i refers to the ith biological model in the appendix. The second and the third columns indicate the number of variables and the number of parameters, respectively, and the columns of BP and DV indicate the times for computing border polynomials and discriminant varieties, respectively.

The computations in the cases indicated with * were performed on a Pentium 4 PC with 3 GHz CPU and 2 G RAM. All the other computations were performed on a T2400 laptop with two CPUs 1.83 GHz and 987 MHz and 512 M RAM. The computational times shown in Tables 2–4 are all in seconds. Each computation was repeated three times and the given timing is the average.

The results in Table 2 show that for some simple systems involving a few variables, computing discriminant varieties takes more time than computing border

TABLE 2. Computational times using the methods of Yang–Xia (YX) and Lazard–Rouillier (LR)

Model	No. of vars	No. of pars	Time YX		Time LR	
			BP	Total	DV	Total
1	2	1	0.156	1.071	0.418	0.730
2	2	1	0.175	1.610	0.374	0.598
3	2	1	0.205	0.915	0.589	0.819
4	3	1	0.234	1.042	0.691	0.970
5	3	1	4.131	15.109	1.258	1.571
6	3	1	0.985	3.521	0.787	0.973
7	3	1	>10 000	>10 000	5.165	7.480
8	3	1	>10 000	>10 000	28.915	48.364
9	4	1	1.952	2.843	2.425	2.683
10	4	1	>10 000	>10 000	49.362	55.624
*12	5	1	⋆>10 000	>10 000	⋆862.625	1004.421
14	2	3	30.872	204.844	48.298	241.127
*15	7	3	⋆35.832	42.173	⋆58.756	61.425

⋆ For these experiments, only partial results can be obtained.

polynomials. However, for all the systems involving only one parameter, the total computational time for solving the stability problems using DV + RS is less than that using DISCOVERER. The main reason may be that the algorithm of real solution isolation used in DISCOVERER is not as fast as that used in RS. For some complex systems involving more variables, the advantage of the method of discriminant varieties is obvious. There are two systems (Models 14 and 15) in our test suite that involve more than one parameter. For these two systems, DISCOVERER is more efficient than DV + RS.

Next, we compare the effect of step 3′ versus step 3 in the general approach described in Section 5. As for systems of dimension 2 (with two variables) the number of Hurwitz determinants and the number of polynomials in the bifurcation conditions are the same (both equal to 2), our experiments have been done only for systems of dimension > 2. Table 3 provides the timings for the entire computations of stability analysis using steps 3 and 3′.

Note that the bifurcation conditions only involve two polynomials, whereas the Routh–Hurwitz criterion is given by n Hurwitz determinants (where n is the number of variables). When $n > 3$, the computation using step 3′ instead of step 3 should be faster. The results in Table 3 show the difference of computational times using the bifurcation conditions (according to step 3′) and the Routh–Hurwitz criterion (according to step 3). Nevertheless, except for Model 13 the gain of using the bifurcation conditions is not very significant.

Finally, we compare the two stability criteria: Routh–Hurwitz's criterion and Liénard–Chipart's criterion. For systems of dimension < 5, the two criteria are

TABLE 3. Computational times using step 3′ vs. step 3

Model	No. of vars	No. of pars	Step 3		Step 3′	
			DV	Total	DV	Total
4	3	1	0.691	0.970	0.672	0.954
5	3	1	1.258	1.571	1.085	1.401
6	3	1	0.787	0.973	0.718	0.904
7	3	1	5.165	7.480	5.002	7.298
8	3	1	28.915	48.364	28.895	44.263
10	4	1	49.362	55.624	43.920	48.315
11	5	1	6.983	7.780	4.829	5.642
*13	5	1	>10 000	>10 000	1584.629	3885.813

coincident, so we only consider systems of dimension ≥ 5. Here we use Lazard–Rouillier's method according to step 3′. The timings for the entire computations using the two criteria are given in Table 4.

TABLE 4. Computational times using the criteria of Routh–Hurwitz (RH) and Liénard–Chipart (LC)

Model	No. of vars	No. of pars	Time RH		Time LC	
			DV	Total	DV	Total
11	5	1	4.829	5.642	4.857	5.607
*12	5	1	⋆862.625	1004.421	⋆864.424	1023.323
*13	5	1	1584.629	3885.813	1579.629	3867.231
*15	7	3	⋆59.226	62.374	⋆58.756	61.425

From our experiments, we find that the computational time also depends on the number of steady states and the number of unstable steady states. If the number of unstable steady states is large, then the computation using Liénard–Chipart's criterion may slow down, because in this case the four sets of conditions in the criterion all have to be verified. If we need to verify only one set of conditions to get a sufficient result, then the computation is obviously faster (than the computation using Routh–Hurwitz's criterion).

Theoretically speaking, for systems of higher dimension, verifying Liénard–Chipart's criterion should be much easier than verifying Routh–Hurwitz's and the advantage of Liénard–Chipart's criterion may become clear. However, the computation for higher-dimensional systems is difficult and beyond our current reach in any case, so we cannot provide experimental evidences to support our theoretical observation.

10. Conclusion

The approach of Wang and Xia [46] for stability analysis of biological systems uses triangular sets, real solution classification, and Routh–Hurwitz's stability criterion. In this paper, we have shown how this approach may be improved, extended, and generalized by making use of Gröbner bases, quantifier elimination (by partial CAD), and discriminant varieties, as well as the stability criterion of Liénard and Chipart. The applicability of the approach to the analysis of stability and Hopf bifurcations has been illustrated by using a class of self-assembling micelle systems with chemical sinks. We have also demonstrated the feasibility of the CAD method for stability analysis and the high efficiency of the method of discriminant varieties by experimental results with comparison for a number of biological models taken from the literature.

As CAD-based methods are designed mainly for real quantifier elimination and are well known to have high computational complexity, they can be applied to stability analysis only for biological systems involving a few (say, less than 5) parameters and variables. Without modification, the CAD method is not very suitable for determining the conditions for a biological system to have a prescribed number of (stable) steady states. However, the CAD method may serve as a convenient device to verify the correctness and completeness of established stability conditions.

Our experiments with 13 biological systems show that Lazard–Rouillier's method of discriminant varieties is a powerful tool for algebraic analysis of stability and bifurcations. The method is similar but computationally superior, in the case where there is only one parameter, to Yang–Xia's method of real solution classification used initially in [46]. However, in the presence of several parameters Yang–Xia's method may be more efficient than Lazard–Rouillier's.

The use of the bifurcation conditions as suggested by Chen [9] may slightly improve the general approach of Wang and Xia. The two criteria of Routh–Hurwitz and Liénard–Chipart perform similarly for biological systems of lower dimension. The latter is expected to have a better performance for systems of higher dimension.

References

[1] Anai, H.: Algebraic methods for solving real polynomial constraints and their applications in biology. In: *Proceedings of the First International Conference on Algebraic Biology* (AB 2005) (Tokyo, Japan, November 28–30, 2005), pp. 139–147. Universal Academy Press, Inc., Tokyo (2005).

[2] Angeli, D., Ferrell, J. E. Jr., Sontag, E. D.: Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems. *Proc. Nat. Acad. Sci. USA* **101**: 1822–1827 (2004).

[3] Aubry, P., Lazard, D., Moreno Maza, M.: On the theories of triangular sets. *J. Symb. Comput.* **28**: 105–124 (1999).

[4] Aubry, P., Moreno Maza, M.: Triangular sets for solving polynomial systems: A comparative implementation of four methods. *J. Symb. Comput.* **28**: 125–154 (1999).

[5] Ball, R., Haymet, A. D. J.: Bistability and hysteresis in self-assembling micelle systems: Phenomenology and deterministic dynamics. *Phys. Chem. Chem. Phys.* **3**: 4753–4761 (2001).

[6] Brown, C. W., Hong, H.: QEPCAD — Quantifier elimination by partial cylindrical algebraic decomposition. http://www.cs.usna.edu/~qepcad/B/QEPCAD.html (2004).

[7] Bruggeman, F. J., Boogerd, F. C., Hornberg, J. J., Lankelma, J., Somsen, O. J. G., Westerhoff, H. V.: Is the signal transduction network emanating from the EGF receptor bistable in vivo? In: *The 9th Meeting of the International Study Group of Bio-ThermoKinetics* (BTK), http://www0.sun.ac.za/biochem/btk/. Stellenbosch, South Africa (2000).

[8] Buchberger, B.: Gröbner bases: An algorithmic method in polynomial ideal theory. In: *Multidimensional Systems Theory* (N. K. Bose, ed.), pp. 184–232. Reidel, Dordrecht (1985).

[9] Chen, C.: Algebraic analysis of stability for biological systems and the implemetation of a software pakage (in Chinese). Master thesis, Peking University, China (2006).

[10] Cinquin, O., Demongeot, J.: Positive and negative feedback: Striking a balance between necessary antagonists. *J. Theor. Biol.* **216**(2): 229–241 (2002).

[11] Collins, G. E.: Quantifier elimination for real closed fields by cylindrical algebraic decomposition. In: *Proceedings of the Second GI Conference on Automata Theory and Formal Languages* (H. Barkhage, ed.), LNCS **33**, pp. 134–183. Springer, Berlin Heidelberg (1975).

[12] Collins, G. E., Hong, H.: Partial cylindrical algebraic decomposition for quantifier elimination. *J. Symb. Comput.* **12**: 299–328 (1991).

[13] El Kahoui, M., Weber, A.: Deciding Hopf bifurcations by quantifier elimination in a software-component architecture. *J. Symb. Comput.* **30**: 161–179 (2000).

[14] Emiris, I. Z., Mourrain, B.: Matrices in elimination theory. *J. Symb. Comput.* **28**: 3–44 (1999).

[15] Faugère, J.-C.: A new efficient algorithm for computing Gröbner bases (F_4). *J. Pure Appl. Algebra* **139**: 61–88 (1999).

[16] Faugère, J.-C., Gianni, P., Lazard, D., Mora, T.: Efficient computation of zero-dimensional Gröbner bases by change of ordering. *J. Symb. Comput.* **16**: 329–344 (1993).

[17] Hong, H., Liska, R., Steinberg, S.: Testing stability by quantifier elimination. *J. Symb. Comput.* **24**: 161–187 (1997).

[18] Hurwitz, A.: Über die Bedingungen, unter welchen eine Gleichung nur Wurzeln mit negativen reellen Theilen besitz. *Math. Ann.* **46**: 273–284 (1895). English translation: On the conditions under which an equation has only roots with negative real part. In: *Selected Papers on Mathematical Trends in Control Theory* (R. Bellman and R. Kalaba, eds.), pp. 72–82. Dover, New York (1964).

[19] Kalkbrener, M.: A generalized Euclidean algorithm for computing triangular representations of algebraic varieties. *J. Symb. Comput.* **15**: 143–167 (1993).

[20] Kholodenko, B. N.: Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur. J. Biochem.* **267**: 1583–1588 (2000).

[21] Kuznetsov, Y. A.: *Elements of Applied Bifurcation Theory* (2nd edn.). Springer, New York (1998).

[22] Lancaster, P., Tismenetsky, M.: *The Theory of Matrices: With Applications*. Academic Press, London (1985).

[23] Lazard, D.: A new method for solving algebraic systems of positive dimension. *Disc. Appl. Math.* **33**: 147–160 (1991).

[24] Lazard, D.: Solving zero-dimensional systems of equations and inequations, depending on parameters. In: *ECCAD* (East Coast Computer Algebra Day) 2004, http://www.cargo.wlu.ca/eccad2004/. Waterloo, Canada (2004).

[25] Lazard, D., Rouillier, F.: Solving parametric polynomial systems. Technical Report RR-5322, INRIA, France (2004).

[26] Liénard, A., Chipart, M. H.: Sur la signe de la partie réelle des racines d'une équation algébrique. *J. Math. Pure Appl.* **10**: 291–346 (1914).

[27] Markevich, N. I., Hoek, J. B., Kholodenko, B. N.: Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J. Cell Biol.* **164**: 353–359 (2004).

[28] Miller, R. K., Michel, A. N.: *Ordinary Differential Equations*. Academic Press, New York London (1982).

[29] Moroz, G., Rouillier, F.: DV — A Maple package for solving parametric polynomial systems. http://fgbrs.lip6.fr/Software/DV/ (2005).

[30] Niu, W.: Application of quantifier elimination and discriminant varieties to stability analysis of biological systems. In: *Proceeding of the First International Conference on Mathematical Aspects of Computer an Information Sciences* (Beijing, China, July 24–26, 2006) (D. Wang and Z. Zheng, eds.), pp. 243–253. Beihang University, China (2006).

[31] Novák, B., Tyson, J. J.: Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J. Cell Sci.* **106**: 1153–1168 (1993).

[32] Pokhilko, A. V., Ataullakhanov, F. I.: Contact activation of blood coagulation: Trigger properties and hysteresis. *J. Theor. Biol.* **191**: 213–219 (1998).

[33] Pomerening, J. R., Sontag, E. D., Ferrell, J. E. Jr.: Building a cell cycle oscillator: Hysteresis and bistability in the activation of Cdc2. *Nature Cell Biol.* **5**: 346–351 (2003).

[34] Rouillier, F., Zimmermann, P.: Efficient isolation of polynomial's real roots. *J. Comput. Appl. Math.* **162**: 33–50 (2004).

[35] Routh, E. J.: *A Treatise on the Stability of a Given State of Motion — Adams Prize Essay*. Macmillan, New York (1877).

[36] Samoilov, M., Plyasunov, S., Arkin, A. P.: Stochastic amplification and signaling in enzymatic futile cycles through noise-induced bistability with oscillations. *Proc. Natl. Acad. Sci. USA*. **102**: 2310–2315 (2005).

[37] Tyson, J. J., Novák, B.: Regulation of the eukaryotic cell cycle: Molecular antagonism, hysteresis, and irreversible transitions. *J. Theor. Biol.* **210**: 249–263 (2001).

[38] van Kooten, T., de Roos, A. M., Persson, L.: Bistability and an Allee effect as emergent consequences of stage-specific predation. *J. Theor. Biol.* **237**: 67–74 (2005).

[39] Wang, D.: Mechanical manipulation for a class of differential systems. *J. Symb. Comput.* **12**: 233–254 (1991).

[40] Wang, D.: An elimination method for polynomial systems. *J. Symb. Comput.* **16**: 83–114 (1993).

[41] Wang, D.: Decomposing polynomial systems into simple systems. *J. Symb. Comput.* **25**: 295–314 (1998).

[42] Wang, D.: Computing triangular systems and regular systems. *J. Symb. Comput.* **30**: 221–236 (2000).

[43] Wang, D.: *Elimination Methods*. Springer, Wien New York (2001).

[44] Wang, D.: *Elimination Practice: Software Tools and Applications*. Imperial College Press, London (2004).

[45] Wang, D.: Computational polynomial algebra and its biological applications. In: *Proceedings of the First International Conference on Algebraic Biology* (AB 2005) (Tokyo, Japan, November 28–30, 2005), pp. 127–137. Universal Academy Press, Inc., Tokyo (2005).

[46] Wang, D., Xia, B.: Stability analysis of biological systems with real solution classification. In: *Proceedings of the 2005 International Symposium on Symbolic and Algebraic Computation* (ISSAC 2005) (M. Kauers, ed.), pp. 354–361. ACM Press, New York (2005).

[47] Wang, D., Xia, B.: Algebraic analysis of stability for some biological systems. In: *Proceedings of the First International Conference on Algebraic Biology* (AB 2005) (Tokyo, Japan, November 28–30, 2005), pp. 75–83. Universal Academy Press, Inc., Tokyo (2005).

[48] Weispfenning, V.: Quantifier elimination for real algebra — The quadratic case and beyond. *Appl. Algebra Eng. Commun. Comput.* **8**: 85–101 (1997).

[49] Wu, W.-t: *Mathematics Mechanization*. Science Press/Kluwer Academic, Beijing (2000).

[50] Xia, B., Yang, L.: An algorithm for isolating the real solutions of semi-algebraic systems. *J. Symb. Comput.* **34**: 461–477 (2004).

[51] Yang, L., Hou, X.-R., Xia, B.: A complete algorithm for automated discovering of a class of inequality-type theorems. *Sci. China* (Ser. F) **44**: 33–49 (2001).

[52] Yang, L., Xia, B.: Real solution classifications of parametric semi-algebraic systems. In: *Algorithmic Algebra and Logic — Proceedings of the A3L 2005* (A. Dolzmann, A. Seidl, and T. Sturm, eds.), pp. 281–289. Herstellung und Verlag, Norderstedt (2005).

[53] Yang, L., Zhang, J.-Z., Hou, X.-R.: A criterion of dependency between algebraic equations and its applications. In: *Proceedings of the International Workshop on Mathematics Mechanization* (W.-t. Wu and M.-d. Cheng, eds.), pp. 110–134. International Academic Publishers, Beijing (1992).

[54] Zhang, Z., Ding, T., Huang, W., Dong, Z.: *Qualitative Theory of Differential Equations*. American Mathematical Society, Providence, RI (1992).

[55] Zwolak, J. W., Tyson, J. J., Watson, L. T.: Finding all steady state solutions of chemical kinetic models. *Nonlinear Analysis: Real World Applications* **5**: 801–814 (2004).

Appendix. Biological models

Model 1. Bruggeman's bistable core model of a signal transduction network [7] This model may serve as a guide in the search for bistability in the signal transduction networks emanating from the epidermal growth factor receptor. It is described by two differential equations

$$\frac{\mathrm{d}E_1}{\mathrm{d}t} = \frac{V_{f1}(S+E_2)(E_{1t}-E_1)}{E_{1t}-E_1+K_{mf1}} - \frac{V_{1b}E_1}{E_1+K_{m1b}},$$
$$\frac{\mathrm{d}E_2}{\mathrm{d}t} = \frac{V_{f2}(S+E_1)(E_{2t}-E_2)}{E_{2t}-E_2+K_{mf2}} - \frac{V_{2b}E_2}{E_2+K_{m2b}},$$

where S is a real parameter and the other biological constants take the values given in Table 5. In the reversible case, $E_{1t} = 1$.

TABLE 5. Constant values for Model 1

V_{f1}	V_{f2}	V_{1b}	V_{2b}	E_{2t}	K_{mf1}	K_{mf2}	K_{m1b}	K_{m2b}
10	10	10	10	2	0.1	0.1	0.1	0.1

Model 2. Mathematical model of the blood contact activation system [32]

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k_1x(s-x-z) + y(s-x-z) - k_3x,$$
$$\epsilon_1\frac{\mathrm{d}y}{\mathrm{d}t} = x(s-x-z)(z+k_5) - k_6y,$$
$$\epsilon_2\frac{\mathrm{d}z}{\mathrm{d}t} = y + k_9x - k_8z.$$

Since $\epsilon_1 > \epsilon_2$, this model asymptotically approaches to

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k_1x(s-x-z) + y(s-x-z) - k_3x,$$
$$\epsilon_1\frac{\mathrm{d}y}{\mathrm{d}t} = x(s-x-z)(z+k_5) - k_6y,$$

where $z = (y+k_9x)/k_8$, s is a real parameter, and the values for the other biological constants are given in Table 6.

TABLE 6. Constant values for Model 2

k_1	k_3	k_5	k_6	k_8	k_9
0.005	17.5	0.012	0.11	0.01	0.05

Model 3. Markevich–Hoek–Kholodenko's model [27]
This model has been used to describe a dual phosphorylation-dephosphorylation cycle. Its dynamical system has the form

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{M}]}{\mathrm{d}t} &= v_4 - v_1,\\ \frac{\mathrm{d}[\mathrm{M}_p]}{\mathrm{d}t} &= v_1 - v_4 + v_3 - v_2,\\ \frac{\mathrm{d}[\mathrm{M}_{pp}]}{\mathrm{d}t} &= v_2 - v_3,\end{aligned}$$

where the last equation reflects the conservation of mass, $\mathrm{M}_{\mathrm{tot}} = [\mathrm{M}_p]+[\mathrm{M}]+[\mathrm{M}_{pp}]$, v_1, v_2, v_3, and v_4 are the reaction rates described by the kinetics laws

$$\begin{aligned}v_1 &= \frac{k_1^{cat}\cdot[\mathrm{MAPKK}]_{\mathrm{tot}}\cdot[\mathrm{M}]/K_{m1}}{(1+[\mathrm{M}]/K_{m1}+[\mathrm{M}_p]/K_{m2})},\\ v_2 &= \frac{k_2^{cat}\cdot[\mathrm{MAPKK}]_{\mathrm{tot}}\cdot[\mathrm{M}_p]/K_{m2}}{(1+[\mathrm{M}]/K_{m1}+[\mathrm{M}_p]/K_{m2})},\\ v_3 &= \frac{k_3^{cat}\cdot[\mathrm{MKP3}]_{\mathrm{tot}}\cdot[\mathrm{M}_{pp}]/K_{m3}}{(1+[\mathrm{M}_{pp}]/K_{m3}+[\mathrm{M}_p]/K_{m4}+[\mathrm{M}]/K_{m5})},\\ v_4 &= \frac{k_4^{cat}\cdot[\mathrm{MKP3}]_{\mathrm{tot}}\cdot[\mathrm{M}_p]/K_{m4}}{(1+[\mathrm{M}_{pp}]/K_{m3}+[\mathrm{M}_p]/K_{m4}+[\mathrm{M}]/K_{m5})},\end{aligned}$$

$[\mathrm{MAPKK}]_{\mathrm{tot}}$ is a real parameter, and the values of the other biological constants are given in Table 7.

TABLE 7. Constant values for Model 3

k_1^{cat}	k_2^{cat}	k_3^{cat}	k_4^{cat}	K_{m1}	K_{m2}	K_{m3}	K_{m4}	K_{m5}	$[\mathrm{MKP3}]_{\mathrm{tot}}$	$\mathrm{M}_{\mathrm{tot}}$
0.01	15	0.084	0.06	50	500	22	18	86	100	500

Model 4. A stage-structured model of an Allee effect [38]

$$\begin{aligned}\frac{\mathrm{d}J}{\mathrm{d}t} &= \beta A - \frac{J}{(1+J^2)} - \mu_J J,\\ \frac{\mathrm{d}A}{\mathrm{d}t} &= \frac{J}{(1+J^2)} - \mu_A A - AP,\\ \frac{\mathrm{d}P}{\mathrm{d}t} &= P(\varepsilon A - \delta),\end{aligned}$$

where δ is a real parameter and the values of the other biological constants are shown in Table 8.

TABLE 8. Constant values for Model 4

β	μ_J	μ_A	ε
1.2	0.06	0.2	1.0

Model 5. A simple model of the MPF activity in frog egg extracts [55]

$$\begin{aligned}
\frac{\mathrm{d}M}{\mathrm{d}t} &= v'_d(1-D)(C_T-M) + v''_d D(C_T-M) - v'_w(1-W)M - v''_w WM,\\
\frac{\mathrm{d}D}{\mathrm{d}t} &= \frac{v_d M(1-D)}{K_{md}+(1-D)} - \frac{v_{dr}D}{K_{mdr}+D},\\
\frac{\mathrm{d}W}{\mathrm{d}t} &= -\frac{v_w MW}{K_{mw}+W} + \frac{v_{wr}(1-W)}{K_{mwr}+(1-W)},
\end{aligned}$$

where C_T is a real parameter and the other biological constants take the values shown in Table 9.

TABLE 9. Constant values for Model 5

v_d	v_{dr}	v_w	v_{wr}	K_{md}	K_{mdr}	K_{mw}	K_{mwr}	v'_d	v''_d	v'_w	v''_w
2	0.1	2	0.1	0.1	1.0	0.1	1.0	0.017	0.17	0.01	1

Models 6–12. Cinquin–Demongeot's model of multistable switch [10, 47]

$$\begin{aligned}
\frac{\mathrm{d}x_1}{\mathrm{d}t} &= -x_1 + \frac{s}{1+x_2^c+x_3^c+\cdots+x_n^c},\\
\frac{\mathrm{d}x_2}{\mathrm{d}t} &= -x_2 + \frac{s}{1+x_1^c+x_3^c+\cdots+x_n^c},\\
&\cdots\cdots\\
\frac{\mathrm{d}x_n}{\mathrm{d}t} &= -x_n + \frac{s}{1+x_2^c+x_3^c+\cdots+x_{n-1}^c},
\end{aligned} \tag{A.1}$$

where $x_1, \ldots, x_n$ denote the concentrations of n proteins, c is the cooperativity, and $s > 0$ is a constant denoting the strength of unrepressed protein expression, relative to the exponential decay. Let s be a real parameter. We consider the following cases.

Model 6. Cinquin–Demongeot's model (A.1) in the case $n = 3$, $c = 2$.

Model 7. Cinquin–Demongeot's model (A.1) in the case $n = 3$, $c = 3$.

Model 8. Cinquin–Demongeot's model (A.1) in the case $n = 3$, $c = 4$.

Model 9. Cinquin–Demongeot's model (A.1) in the case $n = 4$, $c = 1$.

Model 10. Cinquin–Demongeot's model (A.1) in the case $n = 4$, $c = 2$.

Model 11. Cinquin–Demongeot's model (A.1) in the case $n = 5$, $c = 1$.

Model 12. Cinquin–Demongeot's model (A.1) in the case $n = 5$, $c = 2$.

Model 13. Kholodenko's model [20]
The time-dependent behavior of the MAPK cascade may be described by the following system of differential kinetic equations

$$\begin{aligned}
\frac{\mathrm{d}x_1}{\mathrm{d}t} &= \frac{V_2 x_2}{K_2 + x_2} - \frac{V_1 x_1}{\left(1 + \left(\frac{x_8}{K_I}\right)^n\right)(K_1 + x_1)},\\
\frac{\mathrm{d}x_2}{\mathrm{d}t} &= \frac{V_1 x_1}{\left(1 + \left(\frac{x_8}{K_I}\right)^n\right)(K_1 + x_1)} - \frac{V_2 x_2}{K_2 + x_2},\\
\frac{\mathrm{d}x_3}{\mathrm{d}t} &= \frac{V_6 x_4}{K_6 + x_4} - \frac{k_3 x_2 x_3}{K_3 + x_3},\\
\frac{\mathrm{d}x_4}{\mathrm{d}t} &= \frac{k_3 x_2 x_3}{K_3 + x_3} + \frac{V_5 x_5}{K_5 + x_5} - \frac{k_4 x_2 x_4}{K_4 + x_4} - \frac{V_6 x_4}{K_6 + x_4},\\
\frac{\mathrm{d}x_5}{\mathrm{d}t} &= \frac{k_4 x_2 x_4}{K_4 + x_4} - \frac{V_5 x_5}{K_5 + x_5},\\
\frac{\mathrm{d}x_6}{\mathrm{d}t} &= \frac{V_{10} x_7}{K_{10} + x_7} - \frac{k_7 x_5 x_6}{K_7 + x_6},\\
\frac{\mathrm{d}x_7}{\mathrm{d}t} &= \frac{k_7 x_5 x_6}{K_7 + x_6} + \frac{V_9 x_8}{K_9 + x_8} - \frac{k_8 x_5 x_7}{K_8 + x_7} - \frac{V_{10} x_7}{K_{10} + x_7},\\
\frac{\mathrm{d}x_8}{\mathrm{d}t} &= \frac{k_8 x_5 x_7}{K_8 + x_7} - \frac{V_9 x_8}{K_9 + x_8},
\end{aligned}$$

with the moiety conservation relations

$$\begin{aligned}
[\mathrm{MKKK}]_{\mathrm{total}} &= x_1 + x_2,\\
[\mathrm{MKK}]_{\mathrm{total}} &= x_3 + x_4 + x_5,\\
[\mathrm{MAPK}]_{\mathrm{total}} &= x_6 + x_7 + x_8,
\end{aligned}$$

where x_1 =[MKKK], x_2 =[MKKK-P], x_3 =[MKK], x_4 =[MKK-P], x_5 =[MKK-PP], x_6 =[MAPK], x_7 =[MAPK-P], x_8 =[MAPK-PP], V_1 is a real parameter, and the other biological constants take the values given in Table 10.

Model 14. The Cdc2-cyclin B/Wee1 system [2, 31, 33]

$$\begin{aligned}
\frac{\mathrm{d}x}{\mathrm{d}t} &= \alpha_1(1 - x) - \frac{\beta_1 x (vy)^{\gamma_1}}{K_1 + (vy)^{\gamma_1}},\\
\frac{\mathrm{d}y}{\mathrm{d}t} &= \alpha_2(1 - y) - \frac{\beta_2 y x^{\gamma_2}}{K_2 + x^{\gamma_2}},
\end{aligned}$$

where v, K_1, K_2 are real parameters and the other biological constants take the values given in Table 11.

TABLE 10. Constant values for Model 13

n	K_I	K_1	V_2	K_2	k_3	K_3	k_4
1	9	10	0.25	8	0.025	15	0.025
K_4	V_5	K_5	V_6	K_6	k_7	K_7	k_8
15	0.75	15	0.75	15	0.025	15	0.025
K_8	V_9	K_9	V_{10}	K_{10}	$[\mathrm{MKKK}]_{\mathrm{total}}$	$[\mathrm{MKK}]_{\mathrm{total}}$	$[\mathrm{MAPK}]_{\mathrm{total}}$
15	0.5	15	0.5	15	100	300	300

TABLE 11. Constant values for Model 14

γ_1	γ_2	α_1	α_2	β_1	β_2
4	4	1	1	200	10

Model 15. The model of a chemical reaction (communicated by Eduardo D. Sontag from Rutgers University, see also [36])

$$\begin{aligned}
\frac{\mathrm{d}n}{\mathrm{d}t} &= -k_1 n^2 + k_{-1} ne - k_2 n + k_{-2} e,\\
\frac{\mathrm{d}e}{\mathrm{d}t} &= -k_3 se + k_{-3} c_1 + k_4 c_1 + k_1 n^2 - k_{-1} ne + k_2 n - k_{-2} e,\\
\frac{\mathrm{d}s}{\mathrm{d}t} &= -k_3 se + k_{-3} c_1 + k_6 c_2,\\
\frac{\mathrm{d}c_1}{\mathrm{d}t} &= k_3 se - k_{-3} c_1 - k_4 c_1,\\
\frac{\mathrm{d}p}{\mathrm{d}t} &= k_4 c_1 - k_5 pf + k_{-5} c_2,\\
\frac{\mathrm{d}c_2}{\mathrm{d}t} &= k_5 pf - k_{-5} c_2 - k_6 c_2,\\
\frac{\mathrm{d}f}{\mathrm{d}t} &= -k_5 pf + k_{-5} c_2 + k_6 c_2,
\end{aligned}$$

under the conservation laws

$$e + n + c_1 = \alpha, \quad f + c_2 = \beta, \quad s + c_1 + c_2 + p = \gamma,$$

where α, β, γ are real parameters. For the sake of simplicity, we take 1 for all the constants k_i.

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